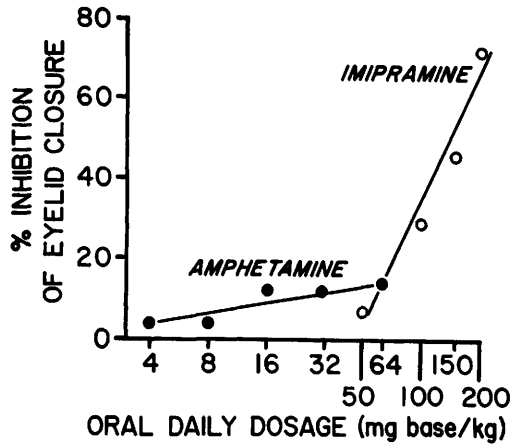

FIG. 1. Inhibition of reserpine eyelid closure in mice by pretreatment for 2 days with amphetamine or imipramine. Challenge with reserpine (2.5 mg/kg i.p.) on third day.

Discussion. Pretreatment with imipramine, an "antidepressant", or with amphetamine, a "stimulant", for at least 2 consecutive days, followed by challenge with reserpine 24 hours after the last drug treatment, produced a differential antagonism to reserpine eyelid closure; only imipramine produced appreciable antagonism. On the other hand, with single administration of the drugs followed by reserpine challenge one hour later, both amphetamine and imipramine were equally active, but higher doses of imipramine were required. These results indicate that the antagonism to reserpine eyelid closure resulting from imipramine lasts longer than that from amphetamine. This difference is similar to differences seen with these compounds in man; amphetamine is a shorter acting drug than imipramine.*

Summary. A differentiation of imipramine, an antidepressant drug, from amphetamine, a stimulant, in the antireserpine eyelid closure test has been described; after administration for 2 days, and reserpine challenge on the third day, imipramine was considerably more active than amphetamine in antagonizing eyelid closure in mice.

Acknowledgement is made to Anne Gillen for statistical evaluation of our data and to Geigy Pharmaceuticals Company for a supply of imipramine.

* Possibly the difference of effects observed by us is indicative of a different mechanism of action.

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Streptokinase Induced Lysinemethylesterase Activity of Human Euglobulin.* (27355)

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When streptokinase (SK) reacts with human plasma, or fractions which contain plasminogen, several types of enzymatic activity are produced. The present concept of SK action attributes each of these activities to the interaction between SK and the single proenzyme, plasminogen. This concept stems from a number of studies(1-5). These indicate that, following SK activation of human plasminogen preparations, activity toward

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separate substrates is produced in the same ratio as in less pure preparations; and specific inhibition procedures affect each activity similarly. Conflicting data indicating that proactivator and arginine esterase activity result from the activation of separate materials have, however, been presented by Lassen(6).

In the present study, SK-induced lysinemethylesterase activity of the euglobulin fraction of human plasma is determined in a large number of individuals. Components of the total activity thus produced are characterized by the effect of two inhibition procedures. It is demonstrated that these components are present in a wide range of ratios to one another and are affected in a different manner by heating, suggesting that they result from the activation of more than one proenzyme.

Materials. Lysinemethylester (LME) was obtained as the dihydrochloride from Nutritional Biochemicals Co., Cleveland, Ohio. A .05 M solution in .025 M potassium phosphate pH 7 buffer in .024 N NaOH was prepared daily.

Streptokinase (SK) Varidase, Lederle was utilized. An SK preparation free of ribonuclease gave identical results.

Soy bean trypsin inhibitor (SBTI) 5× crystallized material was obtained from Nutritional Biochemicals Co.

Methods. Obtaining of specimens. Blood specimens obtained from fasting subjects were placed into ice-enclosed tubes with potassium citrate or sodium versenate anticoagulant and maintained at 4°C. Euglobulin was prepared by the method of Kowalski(7).

Determination of esterase activity. The procedure employed is a modification of that described by Sherry(8). Three ml of LME solution were placed into each of 3 test tubes containing the euglobulin of 0.5 ml plasma. After the protein had been dissolved, the 3 tubes were pooled, 0.5 ml of LME solution were added, and 3 ml of the resultant solution were placed into each of 3 tubes. To the first was added 0.5 ml of buffer, to the second 0.5 ml of SK dissolved in buffer to give a final concentration in the reaction mixture of 286 units/ml, and to the third 0.5 ml of SK and SBTI dissolved in buffer to give a final concentration of SK of 286 u/ml and of SBTI of 286 γ/ml. After incubation at 37°C for 60 minutes, 1.0 ml from each tube was added to 1.0 ml of distilled water and one drop of octyl alcohol. Titration to pH 5.2 was carried out with .05 N HCl by use of a microburette calibrated to .001 ml with continuous stirring by bubbling air. Total SK-induced esterase activity was determined as the micromoles of acid added to the aliquot of the first tube less that added to the second. The activity resistant to SBTI was determined as the micromoles of acid added to the aliquot of the first less that added to the third. Duplicate determinations agreed within .02 micromoles. Only esterase activity due to SK activation was measured.

To determine the esterase activity persisting after precipitation at pH 2 in 2 M NaCl, the euglobulin of 0.5 ml of plasma was dissolved in 1.0 ml glycerol, 0.5 ml of 0.75% casein, and 0.5 ml of SK 2000 u/ml phosphate buffer. Glycerol was added to prevent autodigestion of the plasmin(9). Incubation for 3 minutes was terminated by addition of 10 ml of 2 M NaCl in .01 N HCl. Casein was found to be necessary for development of adequate precipitate for quantitative recovery, but did not otherwise alter esterase activity. After the suspension had been thoroughly mixed and permitted to stand for 10 minutes, it was centrifuged and the supernatant decanted. The precipitate was dissolved in 3.5 ml of LME in pH 7 phosphate buffer. At time 0, and after incubation at 37° for 60 minutes, 1.0 ml was titrated to pH 5.2 as described above.

Esterase activity was determined as the micromoles of acid added at time 0 less that added to the 60-minute specimen. The activity of a blank prepared by similarly treating a mixture of glycerol, casein and SK was subtracted.

Results. Characteristics of the esterase activity. Fig. 1A indicates the first order reaction kinetics of the SK-induced esterase activity for up to 180 minutes utilizing varying amounts of human euglobulin. Fig. 1B indicates that the reaction rate is independent of SK concentration for a range from half to double that utilized. The validity of this

method for determining the activity of the SK-activated material which had been precipitated at pH 2 in 2 M NaCl is indicated in Fig. 1C. Fig. 1D indicates that the inhibition produced by SBTI was constant over a wide range of concentrations of that inhibitor.

Measurement of SK-induced activity. Total SK-induced esterase activity of human euglobulin was measured in 70 subjects and ranged from 2.4 to 10.4 μmoles/hour. The

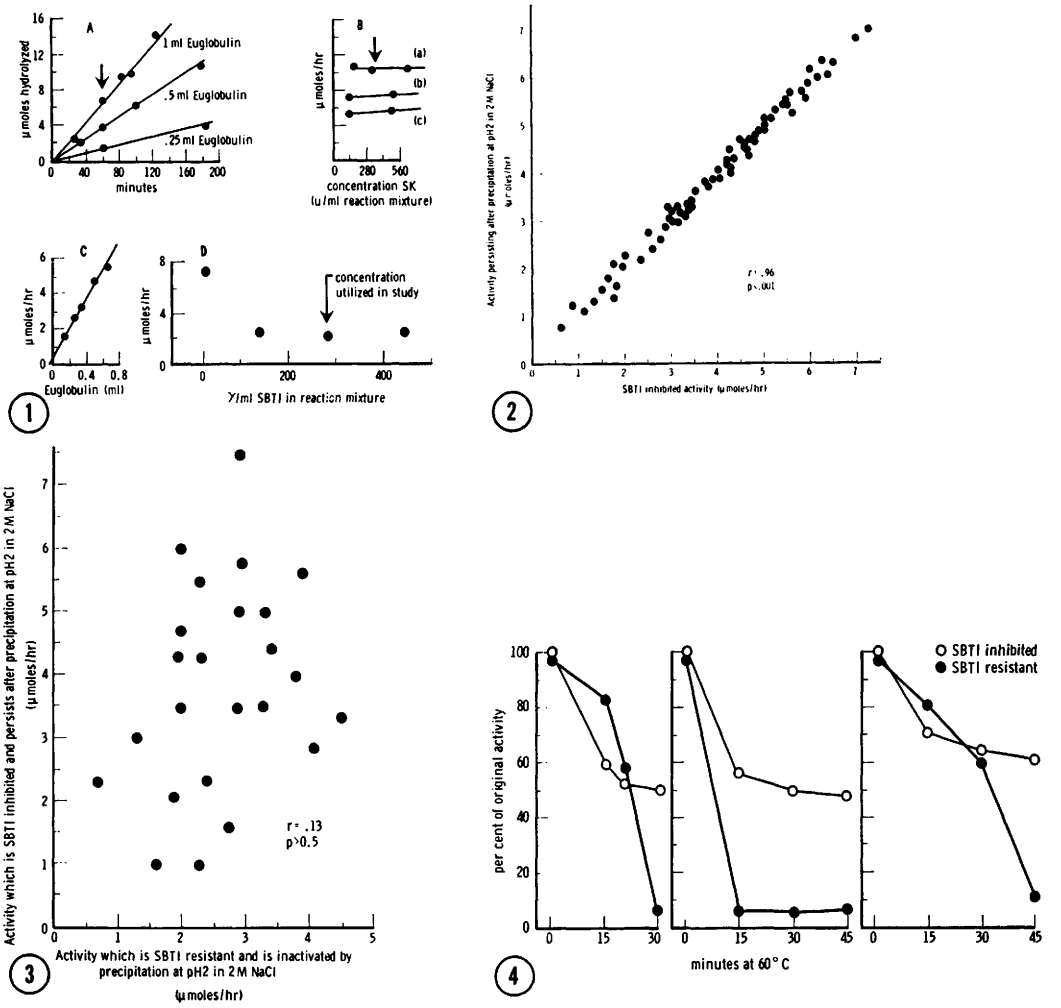

FIG. 1. Kinetics of SK-induced esterase reactions: A) First order reaction kinetics were observed for up to 180 min. at one-half and double the amount of euglobulin utilized in the reported results. B) Reaction rate is independent of SK concentration for a range one-half to double that employed. Arrows indicate time of incubation and SK concentration employed in the reported results. C) Linear relationship between amount of euglobulin and esterase activity following activation by SK and precipitation at pH 2 in 2 M NaCl. D) Degree of inhibition by SBTI independent of concentration from double to half that utilized in this study.

FIG. 2. Relationship between SBTI-sensitive activity and that resistant to precipitation at pH 2 in 2 M NaCl. The close correlation between the activity with each of these characteristics is indicated in 70 euglobulin specimens.

FIG. 3. Relationship between component of total SK-induced activity which is SBTI inhibited and which persists after precipitation at pH 2 in 2 M NaCl and that with the opposite characteristics. Lack of correlation between these 2 fractions is indicated in 25 euglobulin specimens.

FIG. 4. Effect of heating at 60°C on SBTI-inhibited and resistant components of SK-induced esterase activity: The different pattern of inactivation of these 2 fractions by heating at 60°C for up to 45 min. is indicated utilizing 3 specimens of euglobulin.

close agreement between the activity inhibited by SBTI and that persisting after precipitation at pH 2 in 2 M NaCl in each of the specimens tested is indicated in Fig. 2. The ratio between the activity with these characteristics and that activity resistant to SBTI and inactivated by precipitation at pH 2 in 2 M NaCl ranged from 0.5 to 3.0. The absence of correlation between these components of the total activity is evident from Fig. 3.

Effect of heating at 60°C. Euglobulin specimens were dissolved in LME buffer solution. At time 0, and at intervals after incubation at 60°C specimens were removed and total SK-induced activity and that resistant to SBTI were measured. Fig. 4 demonstrates the difference in inactivation between the SBTI resistant and sensitive components in the euglobulin in 3 specimens.

Discussion. Total SK-induced lysinemethylesterase activity of human euglobulin has been shown to consist of 2 components, one of which is inhibited by SBTI and persists after precipitation at pH 2 in 2 M NaCl, and a second on which these procedures have an opposite effect.

Several findings indicate that the apparent separation of total activity into 2 components reflects inhibition of separate materials rather than incomplete inhibition of a single substance. These include the close agreement between the values obtained utilizing 2 inhibition procedures, the wide range of ratios between them, and differences in heat stability.

Ablondi *et al.*(1) have shown that the apparently independent variations of SK-induced esterase activity toward different substrates(10) reflected differences in the solubility of the enzyme at the pH of the reaction mixtures utilized, rather than the presence of 2 individual proesterases. The findings of the present study cannot be explained on this basis for several reasons. In each determination the activity was measured in a reaction mixture of the same composition and pH. Furthermore, there was close agreement between values obtained utilizing separate inhibition procedures and varying concentrations of SBTI.

When SK reacts with preparations of human plasma containing plasminogen, 2 known enzymes, plasmin and the SK-plasminogen-(or plasmin(11)) activator complex, are produced. It has been demonstrated that plasmin possesses lysineesterase activity which is inhibited by SBTI and resistant to precipitation at pH 2(9,10), while the SK-plasminogen activator complex activity is affected in an opposite manner by these procedures(10, 12). In previous studies utilizing more highly purified plasminogen preparations(1-4), the constant ratio between these activities has been invoked as evidence that all enzymatic activity induced by SK results from SK-plasminogen interaction. In the present study, however, a wide range of ratios between the activities with each of these properties as well as different heat lability was demonstrated. These findings suggest that this activity results from the presence of more than one proesterase in human euglobulin. Since activity toward only the synthetic substrate lysinemethylester was determined, the relationship between such a proesterase and the fibrinolytic, proteolytic, or activator activity produced by SK is not determined.

Summary. SK-induced lysinemethylesterase activity of human euglobulin has been determined in 70 subjects. Two components of this activity have been defined, one inhibited by SBTI and resistant to precipitation at pH 2 in 2 M NaCl, and the other affected in an opposite manner by these procedures. A wide range of ratios between them and their different heat lability has been demonstrated, suggesting that they result from the interaction between SK and more than one substance.

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The Human Tumor-Egg Host System II. Discovery and Properties of a New Antitumor Agent, Hadacidin.* (27356)

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Dagg *et al.*(1) and Harris(2,3) have described the use of transplantable human tumors growing in embryonated eggs for chemotherapy studies. We have used the human tumor-egg host system to screen for new antitumor agents. Employing this system, we were successful in discovering a new antitumor agent, N-formyl hydroxyaminoacetic acid, designated hadacidin. Its isolation, structure determination, and synthesis are described(4).

Methods. The procedures of transplantation of tumors and testing of agents are based upon those described by Harris(3). Tumor implants were placed on the chorioallantoic membranes of 9-day embryonated eggs. The eggs were incubated 3 to 4 days, and those showing positive "takes" were selected for therapy. Test agents were injected into the yolk sac. Seven days after injection, the eggs were harvested and tumors and embryos from treated and control groups were weighed.

Results were expressed as follows. Ten eggs were sacrificed at time of injection to determine mean weight of the tumor. The value obtained was subtracted from the mean weight of treated and control tumors at time of harvest. Thus, the actual increase in weight of the tumors during the treatment period was determined. The per cent growth retardation ($100 - T/C \times 100$) compares the increase in weight of treated tumors with the increase in weight of control tumors. The per cent growth retardation for embryos was determined in a similar manner.

Primary screening was against the human adenocarcinoma, HAD #1(5). Additional tests were made against the human epidermoid carcinoma, HEP #3(6) and the human bronchogenic carcinoma, A-42(7).†

Microorganisms isolated from various habitats were grown in 250 ml Erlenmeyer flasks containing 50 ml of medium of the following composition: glucose, 4%; corn steep liquor, 1%; Edamine‡ (enzymatic digest of casein), 2%; distilled water to volume. The pH was adjusted to 6.8 with NaOH prior to sterilization. The flasks were incubated at 28°C on a rotary shaker moving at 220 rpm.

After 6 days' incubation, the fermentation broths were clarified by admixing with Super-Cel§ and filtering through a Buchner funnel. The clarified filtrates were sterilized by passage through Selas 03 filters‖ prior to testing. When necessary, dilutions were made with Earle's Balanced Salt Solution (ES) which was also used as diluent for water-soluble solids. One ml of filtrate or appropriate dilution was injected per egg. The control

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‡ Sheffield Chemical Co., Inc., Norwich, N. Y.

§ Johns-Manville, New York.

‖ Selas Filter Corp. of America, Dresher, Pa.